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=> d his

     (FILE 'HOME' ENTERED AT 16:22:28 ON 09 JUL 2001)

     FILE 'REGISTRY' ENTERED AT 16:22:40 ON 09 JUL 2001
L1             1 S 9068-25-1/RN

     FILE 'HCAPLUS' ENTERED AT 16:23:33 ON 09 JUL 2001

     FILE 'REGISTRY' ENTERED AT 16:24:09 ON 09 JUL 2001
                SET SMARTSELECT ON
L2           SEL L1 1- CHEM :       8 TERMS
                SET SMARTSELECT OFF

     FILE 'HCAPLUS' ENTERED AT 16:24:10 ON 09 JUL 2001
L3           283 S L2
L4           229 S L3 AND PD<19980227
                E GENETIC ENGINEER/CT
                E E4=ALL
                E GENETIC ENGINEER/CT
                E E4+ALL
L5             1 S L4 AND GENETIC ENGINEER?
                E MUTATION/CT
                E E3+ALL
L6             4 S L4 AND MUTAT?
                E ALPHA AMYLASE/CT
                E MALTOGENIC AMYLASE/CT
                E AMYLASE/CT
                E E3+ALL
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=> d iall l6 1-4

L6 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:672034 HCAPLUS
DOCUMENT NUMBER: 130:33642
TITLE: Production of human compatible high mannose-type (Man5GlcNAc2) sugar chains in Saccharomyces cerevisiae
AUTHOR(S): Chiba, Yasunori; Suzuki, Misa; Yoshida, Satoshi; Yoshida, Aruto; Ikenaga, Hiroshi; Takeuchi, Makoto; Jigami, Yoshifumi; Ichishima, Eiji
CORPORATE SOURCE: Central Laboratories for Key Technology, KIRIN Brewery Co., Ltd., Kanagawa, 236-0004, Japan
SOURCE: J. Biol. Chem. (**1998**), 273(41), 26298-26304
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
CLASSIFICATION: 3-2 (Biochemical Genetics)
Section cross-reference(s): 16

ABSTRACT:
A yeast mutant capable of producing Man5GlcNAc2 human compatible sugar chains on glycoproteins was constructed. An expression vector for **.alpha.-*****1*** **,2-mannosidase** with the "HDEL" endoplasmic reticulum retention/retrieval tag was designed and expressed in Saccharomyces cerevisiae. An in vitro **.alpha.-1,2-*****mannosidase*** assay and Western blot anal. showed that it was successfully localized in the endoplasmic reticulum. A triple mutant yeast lacking three glycosyltransferase activities was then transformed with an **.*****alpha*** **.-1,2-mannosidase** expression vector. The oligosaccharide structures of carboxypeptidase Y as well as cell surface glycoproteins were analyzed, and the recombinant yeast was shown to produce a series of high mannose-type sugar chains including Man5GlcNAc2. This is the first report of a recombinant S. cerevisiae able to produce Man5GlcNAc2-oligosaccharides, the intermediate for hybrid-type and complex-type sugar chains.

SUPPL. TERM: mannose sugar chain human compatible prodn Saccharomyces; mannosidase expression vector ManGlcNAc prodn recombinant Saccharomyces

INDEX TERM: Glycoproteins (specific proteins and subclasses)
ROLE: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
((Man5GlcNAc2)-contg.; prodn. of human compatible high mannose-type (Man5GlcNAc2) sugar chains in Saccharomyces cerevisiae)

INDEX TERM: Aspergillus phoenicis
Endoplasmic reticulum
(Aspergillus saitoi **.alpha.-1, 2-mannosidase** targeted to ER of Saccharomyces cerevisiae; for prodn. of human compatible high mannose-type (Man5GlcNAc2) sugar chains in S. cerevisiae)

INDEX TERM: Polysaccharides, preparation
ROLE: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(mannose-contg.; prodn. of human compatible high mannose-type (Man5GlcNAc2) sugar chains in Saccharomyces cerevisiae)

INDEX TERM: Genetic engineering
Saccharomyces cerevisiae
(prodn. of human compatible high mannose-type (Man5GlcNAc2) sugar chains in Saccharomyces cerevisiae)

INDEX TERM: Glycosylation (biological)
(prodn. of human compatible high mannose-type (Man5GlcNAc2) sugar chains in Saccharomyces cerevisiae contg.)

INDEX TERM: 9055-06-5, Mannosyltransferase
ROLE: BSU (Biological study, unclassified); BIOL (Biological study)
(**mutations** in OCH1, MNN1, and MNN4 genes for; prodn. of human compatible high mannose-type (Man5GlcNAc2) sugar chains in Saccharomyces cerevisiae contg.)

INDEX TERM: **9068-25-1, .alpha.-1,2-Mannosidase**
ROLE: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(prodn. of human compatible high mannose-type (Man5GlcNAc2) sugar chains in Saccharomyces cerevisiae using)

REFERENCE COUNT: 37

REFERENCE(S):

(1) Abeijon, C; Proc Natl Acad Sci U S A 1989, V86, P6935 HCAPLUS
(2) Camirand, A; J Biol Chem 1991, V266, P15120 HCAPLUS
(3) Cowles, C; EMBO J 1997, V16, P2769 HCAPLUS
(4) Fujita, A; Biochem Biophys Res Commun 1997, V238, P779 HCAPLUS
(5) Fukuda, M; Blood 1989, V73, P84 HCAPLUS
(6) Gaynor, E; J Cell Biol 1994, V127, P653 HCAPLUS
(7) Graham, T; J Cell Biol 1991, V114, P207 HCAPLUS
(8) Herscovics, A; J Biol Chem 1994, V269, P9864 HCAPLUS
(9) Hsu, S; J Histochem Cytochem 1981, V29, P577 MEDLINE
(10) Inoue, T; Biochim Biophys Acta 1995, V1253, P141 HCAPLUS
(11) Ito, H; J Bacteriol 1983, V153, P163 HCAPLUS
(12) Johansen, J; Carlsberg Res Commun 1976, V41, P1 HCAPLUS
(13) Kondo, A; Agric Biol Chem 1990, V54, P2169 HCAPLUS
(14) Kubota, S; J Biochem (Tokyo) 1977, V81, P197 HCAPLUS
(15) Laemmli, U; Nature 1970, V227, P680 MEDLINE
(16) Lal, A; J Biol Chem 1994, V269, P9872 HCAPLUS
(17) Lewis, M; Nature 1990, V348, P162 HCAPLUS
(18) Lussier, M; J Cell Biol 1995, V131, P913 HCAPLUS
(19) Nakanishi-Shindo, Y; J Biol Chem 1993, V268, P26338 HCAPLUS
(20) Peat, S; J Chem Soc 1961, P29 HCAPLUS
(21) Pelham, H; EMBO J 1988, V7, P913 HCAPLUS
(22) Redding, K; J Cell Biol 1991, V113, P527 HCAPLUS
(23) Semenza, J; Cell 1990, V61, P1349 HCAPLUS
(24) Sherman, F; Methods Enzymol 1991, V194, P21 MEDLINE
(25) Sikorski, R; Genetics 1989, V122, P19 HCAPLUS
(26) Spivak, J; Blood 1989, V73, P90 HCAPLUS
(27) Takeuchi, M; Glycobiology 1991, V1, P337 HCAPLUS
(28) Takeuchi, M; Proc Natl Acad Sci U S A 1989, V86, P7819 HCAPLUS
(29) Te Heesen, S; EMBO J 1992, V11, P2071 HCAPLUS
(30) Te Heesen, S; Eur J Cell Biol 1991, V56, P8 HCAPLUS
(31) Towbin, H; Proc Natl Acad Sci U S A 1979, V76, P4350 HCAPLUS
(32) Tremblay, L; Glycobiology 1998, V8, P585 HCAPLUS
(33) Trimble, R; J Biol Chem 1983, V258, P2562 HCAPLUS
(34) Vida, T; J Cell Biol 1990, V111, P2871 HCAPLUS
(35) Wang, J; J Toxicol Environ Health 1981, V8, P639 HCAPLUS
(36) Yip, C; Proc Natl Acad Sci U S A 1994, V91, P2723 HCAPLUS
(37) Yoshida, T; Biochim Biophys Acta 1995, V1263, P159

L6 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:633828 HCAPLUS
DOCUMENT NUMBER: 126:101574
TITLE: N-glycosylation affects endoplasmic reticulum degradation of a **mutated** derivative of carboxypeptidase yscY in yeast
AUTHOR(S): Knop, Michael; Hauser, Nicole; Wolf, Dieter H.
CORPORATE SOURCE: Inst. Biochem., Univ. Stuttgart, Stuttgart, D-70569, Germany
SOURCE: Yeast (**1996**), 12(12), 1229-1238
CODEN: YESTE3; ISSN: 0749-503X
PUBLISHER: Wiley
DOCUMENT TYPE: Journal
LANGUAGE: English
CLASSIFICATION: 10-2 (Microbial, Algal, and Fungal Biochemistry)
Section cross-reference(s): 6

ABSTRACT:
The endoplasmic reticulum (ER) of eukaryotic cells contains a quality control system, that is required for the proteolytic removal of aberrantly folded proteins that accumulate in this organelle. We used genetic and biochem. methods to analyze the involvement of N-glycosylation in the degrdn. of a mutant deriv. of carboxypeptidase yscY in the ER of the yeast Saccharomyces cerevisiae. Our results demonstrate that N-glycosylation of this protein is required for its degrdn. since an unglycosylated species is retained stably in the ER. Cells that were devoid of the ER-processing **.alpha.1,2-mannosidase** showed reduced degrdn. of the glycosylated substrate protein. Disruption of CNE1, a gene encoding a putative yeast homolog for calnexin, did not exhibit any effects on the degrdn. of this substrate protein in vivo. Also, the **.alpha.1,2-*****mannosidase*** -dependent redn. in the degrdn. rate did not show any correlation with the function of the CNE1 gene product. Our results suggest that the ER of yeast contains a glycosylation-dependent quality control system, as has been shown for higher eukaryotic cells.

SUPPL. TERM: glycosylation endoplasmic reticulum degrdn yeast; carboxypeptidase yscY glycosylation degrdn yeast ER; **mutation** degrdn yeast endoplasmic reticulum
INDEX TERM: Endoplasmic reticulum
Glycosylation
Mutation
Protein degradation
(N-glycosylation affects endoplasmic reticulum degrdn. of a **mutated** deriv. of carboxypeptidase yscY in yeast)
INDEX TERM: **9068-25-1, .alpha.-1,2-Mannosidase**
ROLE: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BIOL (Biological study); OCCU (Occurrence)
(N-glycosylation affects endoplasmic reticulum degrdn. of a **mutated** deriv. of carboxypeptidase yscY in yeast)
INDEX TERM: 121257-44-1, Proteinase yscY
ROLE: BPR (Biological process); BIOL (Biological study); PROC (Process)
(N-glycosylation affects endoplasmic reticulum degrdn. of a **mutated** deriv. of carboxypeptidase yscY in yeast)

L6 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:724853 HCAPLUS
DOCUMENT NUMBER: 123:163991

TITLE: Two naturally occurring mouse .**alpha**.-**1,2-mannosidase** IB cDNA clones differ in three point **mutations**. **Mutation** of Phe592 to Ser592 is sufficient to abolish enzyme activity

AUTHOR(S): Schneikert, Jean; Herscovics, Annette
CORPORATE SOURCE: McGill Cancer Center, McGill Univ., Montreal, PQ, H3G 1Y6, Can.

SOURCE: J. Biol. Chem. (**1995**), 270(30), 17736-40
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
CLASSIFICATION: 7-5 (Enzymes)
Section cross-reference(s): 3, 13

ABSTRACT:
In mammalian cells, .**alpha**.-**1,2-** ***mannosidases*** play an essential role in the early steps of N-linked oligosaccharide maturation. We previously reported the isolation of mouse .alpha.-mannosidase IB cDNA clones from a Balb/c 3T3 cDNA library. Clone 4 encodes a type II membrane protein of 641 amino acids with a cytoplasmic tail of 35 amino acids, followed by a transmembrane domain and a large C-terminal catalytic domain, whereas clone 16 encodes only the last 471 amino acids. Their overlapping sequences (from amino acid 152) are identical, except for three point **mutations** that result in three amino acid differences in the catalytic domain of the enzyme (Thr411, Leu468, and Ser592 in clone 4 to Met411, Phe468, and Phe592 in clone 16, resp.). Both sequences could be amplified by polymerase chain reaction using templates of cDNAs derived from colon and brain of CD1 mice and from L cells derived from the C3H/An mouse, indicating that both are natural isoforms found in two inbred and one outbred mouse strains. When expressed in COS7 cells as a secreted protein A fusion protein, the catalytic domain of clone 16 displays .**alpha**.-**1,2-mannosidase** activity using [3H]mannose-labeled Man9GlcNAc as substrate, but the corresponding region of clone 4 is poorly secreted under identical conditions. The contribution of each point ***mutation*** to this differential secretion and enzyme activity of the two fusion proteins was assessed by testing the six recombinants corresponding to all the possible sequence permutations. **Mutation** of Phe592 to Ser592, as found in clone 4, is sufficient to abolish .**alpha**.-***1*** ,**2-mannosidase** activity, whereas **mutation** of Met411 to Thr411 or of Phe468 to Leu468 affects secretion with relatively little effect on enzyme activity. Phe592 is part of a highly conserved region that seems important for enzyme activity of class 1 .**alpha**.-**1,2-mannosidases**.

SUPPL. TERM: mouse alpha mannosidase point **mutation** difference
INDEX TERM: **Mutation**
(**mutation** of Phe592 to Ser592, as in clone 4, is sufficient to abolish mouse .**alpha**.-**1,2-mannosidase** activity, whereas **mutation** of Met411 to Thr411 or of Phe468 to Leu468 affects secretion with little effect on enzyme activity)
INDEX TERM: Brain
Polymerase chain reaction
(two mouse .**alpha**.-**1,2-mannosidase** IB cDNA clones could be amplified by PCR using templates of cDNAs derived from colon and brain of CD1 mice and from L cells derived from the C3H/An mouse, indicating that both are natural isoforms)
INDEX TERM: Mouse
(two naturally occurring mouse .**alpha**.-**1,2-mannosidase** IB cDNA clones differ in three point **mutations**. **Mutation** of Phe592 to Ser592 is sufficient to

abolish enzyme activity)

INDEX TERM: Animal cell line
(COS-7, when **.alpha.-1,2-mannosidase** IB cDNA is expressed in COS7 cells as a secreted protein A fusion protein, the catalytic domain of clone 16 displays **.alpha.-1,2-mannosidase** activity but the corresponding region of clone 4 is poorly secreted)

INDEX TERM: Animal cell line
(L, two mouse **.alpha.-1,2-mannosidase** IB cDNA clones could be amplified by PCR using templates of cDNAs derived from colon and brain of CD1 mice and from L cells derived from the C3H/An mouse, indicating that both are natural isoforms)

INDEX TERM: Intestine
(colon, two mouse **.alpha.-1,2-mannosidase** IB cDNA clones could be amplified by PCR using templates of cDNAs derived from colon and brain of CD1 mice and from L cells derived from the C3H/An mouse, indicating that both are natural isoforms)

INDEX TERM: Deoxyribonucleic acids
ROLE: PRP (Properties)
(complementary, two naturally occurring mouse **.alpha.-1,2-mannosidase** IB cDNA clones differ in three point **mutations. Mutation** of Phe592 to Ser592 is sufficient to abolish enzyme activity)

INDEX TERM: Proteins, specific or class
ROLE: BAC (Biological activity or effector, except adverse); BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(fusion products, protein A; when **.alpha.-1,2-mannosidase** IB cDNA is expressed in COS7 cells as a secreted protein A fusion protein, the catalytic domain of clone 16 displays **.alpha.-1,2-mannosidase** activity but the corresponding region of clone 4 is poorly secreted)

INDEX TERM: **Mutation**
(point, two naturally occurring mouse **.alpha.-1,2-mannosidase** IB cDNA clones differ in three point **mutations. Mutation** of Phe592 to Ser592 is sufficient to abolish enzyme activity)

INDEX TERM: Biological transport
(secretion, **mutation** of Phe592 to Ser592, as in clone 4, is sufficient to abolish mouse **.alpha.-1,2-mannosidase** activity, whereas **mutation** of Met411 to Thr411 or of Phe468 to Leu468 affects secretion with little effect on enzyme activity)

INDEX TERM: 63-91-2, Phenylalanine, biological studies
ROLE: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(592; two naturally occurring mouse **.alpha.-1,2-mannosidase** IB cDNA clones differ in three point **mutations. Mutation** of Phe592 to Ser592 is sufficient to abolish enzyme activity)

INDEX TERM: **9068-25-1, .alpha.-1,2-Mannosidase**
ROLE: PRP (Properties)
(IB; two naturally occurring mouse **.alpha.-1,2-mannosidase** IB cDNA

clones differ in three point **mutations**.
Mutation of Phe592 to Ser592 is sufficient to
abolish enzyme activity)

INDEX TERM: 63-68-3, Methionine, biological studies
ROLE: BAC (Biological activity or effector, except adverse);
BIOL (Biological study)
(**mutation** of Phe592 to Ser592, as in clone 4,
is sufficient to abolish mouse .**alpha**.-
1,2-mannosidase activity,
whereas **mutation** of Met411 to Thr411 or of
Phe468 to Leu468 affects secretion with little effect on
enzyme activity)

INDEX TERM: 61-90-5, Leucine, biological studies 72-19-5, Threonine,
biological studies
ROLE: BSU (Biological study, unclassified); BIOL (Biological
study)
(**mutation** of Phe592 to Ser592, as in clone 4,
is sufficient to abolish mouse .**alpha**.-
1,2-mannosidase activity,
whereas **mutation** of Met411 to Thr411 or of
Phe468 to Leu468 affects secretion with little effect on
enzyme activity)

INDEX TERM: 56-45-1, Serine, biological studies
ROLE: BSU (Biological study, unclassified); BIOL (Biological
study)
(two naturally occurring mouse .**alpha**.-
1,2-mannosidase IB cDNA
clones differ in three point **mutations**.
Mutation of Phe592 to Ser592 is sufficient to
abolish enzyme activity)

L6 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1980:108565 HCAPLUS
DOCUMENT NUMBER: 92:108565
TITLE: Detection of cystic fibrosis homozygotes and heterozygotes with plasma
AUTHOR(S): Hirani, S.; Winchester, B. G.
CORPORATE SOURCE: Dep. Biochem., Queen Elizabeth Coll., London, W8 7AH, Engl.
SOURCE: Lancet (**1979**), 2(8148), 906-7
CODEN: LANCAO; ISSN: 0023-7507
DOCUMENT TYPE: Journal
LANGUAGE: English
CLASSIFICATION: 14-3 (Mammalian Pathological Biochemistry)
ABSTRACT:
An increase in the proportion of the thermolabile intermediate .**alpha**.-**mannosidase** (**I**) in cystic fibrosis (CF) plasma was manifested as a decreased thermostability of total I at pH 5.4, the pH used by Hosli and E. Vogt (1979) in a method for detection of CF heterozygotes and homozygotes. Alternatively, a **mutation** in the normally stable acidic I leading to a less stable form would be reflected as a decreased stability of total I at pH 5.4, esp. if the proportion of acidic I was raised in CF. The use of a differential assay for the 2 forms of I may thus be of value in diagnosis of CF, and may also provide an indication of the basic defect in CF.

SUPPL. TERM: cystic fibrosis alpha mannosidase plasma
INDEX TERM: Cystic fibrosis
(mannosidase of blood plasma in relation to diagnosis of)
INDEX TERM: Blood plasma
(mannosidase of, cystic fibrosis diagnosis in relation to)
INDEX TERM: 9025-42-7
ROLE: BIOL (Biological study)
(of blood plasma, cystic fibrosis diagnosis in relation

to)

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Search Results -

Terms	Documents
(l9 or l8 or l7 or l6 or l5) and l4	1

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

Refine Search: (l9 or l8 or l7 or l6 or l5) and l4 Clear

Search History

Today's Date: 7/9/2001

DB Name	Query	Hit Count	Set Name
USPT,PGPB	(l9 or l8 or l7 or l6 or l5) and l4	1	L10
USPT,PGPB	(((530/350)!.CCLS.))	5482	L9
USPT,PGPB	(((435/471)!.CCLS.))	437	L8
USPT,PGPB	(((435/252.31)!.CCLS.))	499	L7
USPT,PGPB	(((435/202)!.CCLS.))	232	L6
USPT,PGPB	((435/69.1)!.CCLS.)	6218	L5
USPT,PGPB	l3 and @ad<19980227	5	L4
USPT,PGPB	l2 and l1	5	L3
USPT,PGPB	genetic engineer$ or mutant$1 or mutation$1	34865	L2
USPT,PGPB	alpha 1,2 Mannosidase or alpha Mannosidase I or 1,2 alpha D Mannosidase or E C 3.2.1.113 or Exo alpha 1,2 mannanase or Exo 1,2 alpha mannosidase or Mannose 9 processing alpha mannosidase	13	L1

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☐ 1. Document ID: US 5871997 A

L4: Entry 1 of 5 File: USPT Feb 16, 1999

US-PAT-NO: 5871997
DOCUMENT-IDENTIFIER: US 5871997 A

TITLE: Methods and compositions for protecting retroviral vector particles and producer cells from inactivation by complement via reduction of the expression or recognition of galactose alpha (1,3) galactosyl epitopes

DATE-ISSUED: February 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rother; Russell P.	Cheshire	CT	N/A	N/A
Rollins; Scott A.	Monroe	CT	N/A	N/A
Fodor; William L.	New Haven	CT	N/A	N/A
Springhorn; Jeremy P.	Cheshire	CT	N/A	N/A
Squinto; Stephen P.	Bethany	CT	N/A	N/A

US-CL-CURRENT: 435/235.1; 435/238, 435/239, 435/325

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 5834251 A

L4: Entry 2 of 5 File: USPT Nov 10, 1998

US-PAT-NO: 5834251
DOCUMENT-IDENTIFIER: US 5834251 A

TITLE: Methods of modifying carbohydrate moieties

DATE-ISSUED: November 10, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maras; Marleen	Gentbrugge	N/A	N/A	BEX
Contreras; Roland	Merelbeke	N/A	N/A	BEX

US-CL-CURRENT: 435/71.1; 435/171, 435/68.1, 435/69.1, 435/72, 435/85, 435/97, 435/99

Full | Title | Citation | Front | Review | Classification | Date | Reference | KWIC | Draw Desc | Image

☐ 3. Document ID: US 5770405 A

L4: Entry 3 of 5 File: USPT Jun 23, 1998

US-PAT-NO: 5770405
DOCUMENT-IDENTIFIER: US 5770405 A

TITLE: Isolation and composition of novel glycosidases

DATE-ISSUED: June 23, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wong-Madden; Sharon T.	Newburyport	MA	N/A	N/A
Guthrie; Ellen P.	Andover	MA	N/A	N/A
Taron; Christopher H.	Champaign	IL	N/A	N/A
Landry; David	Essex	MA	N/A	N/A
Guan; Chudi	Wenham	MA	N/A	N/A
Robbins; Phillips W.	Beverly	MA	N/A	N/A

US-CL-CURRENT: 435/74; 435/200, 435/208, 435/910, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

☐ 4. Document ID: US 5721121 A

L4: Entry 4 of 5 File: USPT Feb 24, 1998

US-PAT-NO: 5721121
DOCUMENT-IDENTIFIER: US 5721121 A

TITLE: Mammalian cell culture process for producing a tumor necrosis factor receptor immunoglobulin chimeric protein

DATE-ISSUED: February 24, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Etcheverry; Tina	Berkeley	CA	N/A	N/A
Ryll; Thomas	San Mateo	CA	N/A	N/A

US-CL-CURRENT: 435/69.7; 435/325, 435/328, 435/358, 435/361, 530/387.3, 530/395

Full | Title | Citation | Front | Review | Classification | Date | Reference KWIC | Draw Desc | Image

☐ 5. Document ID: US 5705364 A

L4: Entry 5 of 5 File: USPT Jan 6, 1998

US-PAT-NO: 5705364
DOCUMENT-IDENTIFIER: US 5705364 A

TITLE: Mammalian cell culture process

DATE-ISSUED: January 6, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Etcheverry; Tina	Berkeley	CA	N/A	N/A
Ryll; Thomas	San Mateo	CA	N/A	N/A

US-CL-CURRENT: 435/70.3; 435/375, 435/383, 435/395

Full | Title | Citation | Front | Review | Classification | Date | Reference

KWIC | Draw Desc | Image

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Terms	Documents
13 and @ad<19980227	5

Display 10 Documents, starting with Document: 5

Display Format: CIT Change Format

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Search Results - Record(s) 1 through 1 of 1 returned.

☐ 1. Document ID: US 5834251 A

L10: Entry 1 of 1 File: USPT Nov 10, 1998

US-PAT-NO: 5834251
DOCUMENT-IDENTIFIER: US 5834251 A

TITLE: Methods of modifying carbohydrate moieties

DATE-ISSUED: November 10, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Maras; Marleen	Gentbrugge	N/A	N/A	BEX
Contreras; Roland	Merelbeke	N/A	N/A	BEX

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Alko Group Ltd.	Helsinki	N/A	N/A	FIX	03

APPL-NO: 8/366800
DATE FILED: December 30, 1994

INT-CL: [6] C12P 21/00, C12P 19/18, C12P 1/02
US-CL-ISSUED: 435/71.1; 435/72, 435/85, 435/97, 435/99, 435/69.1, 435/171, 435/68.1
US-CL-CURRENT: 435/71.1; 435/171, 435/68.1, 435/69.1, 435/72, 435/85, 435/97, 435/99
FIELD-OF-SEARCH: 435/71.1, 435/85, 435/97, 435/99, 435/171, 435/72, 435/69.1, 435/68.1

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
4770994	September 1988	Rittenhouse	435/7
5180674	January 1993	Roth	435/288
5272066	December 1993	Bergh et al.	435/97
5324663	June 1994	Lowe	435/320.1

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0 244 234	November 1987	EPX	

OTHER PUBLICATIONS

Burke, J. et al., "The Transmembrane and Flanking Sequences of .beta.1,2-N-Acetylglucosaminytransferase I Specify medial-Golgi Localization," J. Biol. Chem. 267(34):24433-24440 (1992).
Carrez, D. et al., "Heterologous gene expression by filamentous fungi: secretion of human interleukin-6 by Aspergillus nidulans, " Gene 94:147-154 (1990).
Chiba, Y. et al., "The Carbohydrate Moiety of the Acid Carboxypeptidase from Aspergillus saitoi, " Curr. Microbial. 27:281-288 (1993).
Contreras R. et al., "Efficient Kex2-like Processing of a Glucoamyase-Interleukin-6 Fusion Protein by Aspergillus nidulans and Secretion of Mature Interleukin-6," Bio/Technology 9:378-381 (1991).
Dwek, R.A. et al., "Analysis of Glycoprotein-Associated Oligosaccharides," Annu. Rev. Biochem. 62:65-100 (1993).
Harkki, A. et al., "A Novel Fungal Expression System: Secretion of Active Calf Chymosin from the Filamentous Fungus Trichoderma reesei," Bio/Technology 7:596-601 (1989).
Keskar, S.S. et al., "Production and Properties of .alpha.-Mannosidase from Aspergillus sp.," Biotech. Lett. 15(7):685-690 (1993).
Kukuruzinska, M.A. et al., "Protein Glycosylation in Yeast," Ann. Rev. Biochem. 56:915-944 (1987).
Nevalainen, H., "Genetic Improvement of Enzyme Production in Industrially Important Fungal Strains," Presented at the Univ. of Helsinki on Nov. 29, 1985.

Salovuori, I. et al., "Low Molecular Weight High-Mannose Type Glycans in a Secreted Protein of the Filamentous Fungus Trichoderma reesei," Bio/Technology 5:152-156 (1987).
Schachter, Hl., "The `yellow brick road` to branched complex N-glycans," Glycobiology 1(5):453-461 (1991).
Shoemaker, S. et al., "Characterization and Properties of Cellulases Purified From Trichoderma reesei Strain L27," Bio/Technology 1:687-690 (1983).
Stanley, P., "Chinese Hamster Ovary Cell Mutants with Multiple Glycosylation Defects for Production of Glycoproteins with Minimal Carbohydrate Heterogeneity," Molec. Cell. Biol. 9(2):377-383 (1989).
Stanley, P., "Glycosylation engineering ," Glycobiology 2(2):99-107 (1992).
Uusitalo, J.M. et al., "Enzyme production by recombinant Trichoderma reesei strains," J. Biotech. 17:35-50 (1991).
van den Hondel, C. et al., "Heterologous Gene Expression in Filamentous Fungi," in: More Gene Manipulations in Fungi, Bennett et al., eds., pp. 396-428 (1991).

Van Brunt, J., "Fungi: The Perfect Hosts?" Bio/Technology 4:1057-1059 and 1060-1062 (1986).
Varki, A., "Biological roles of oligosaccharides: all of the theories are correct," Glycobiology 3(2):97-130 (1993).
Toone et al, Tetrahedron 45(17): 5365-5422 (1989).

ART-UNIT: 188
PRIMARY-EXAMINER: Rollins; John W.
ASSISTANT-EXAMINER: Prats; Francisco C.
ATTY-AGENT-FIRM: Sterne,Kessler, Goldstein & Fox, p.l.l.c.

ABSTRACT:

The invention is directed to methods of converting high mannose type glycosylation patterns to hybrid or complex type glycosylation patterns.

20 Claims, 16 Drawing figures

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Clear History

1: J Biol Chem 1996 Sep 27;271(39):23836-41

Structural and functional aspects of chloride binding to Alteromonas haloplanctis alpha-amylase.

Feller G, Bussy O, Houssier C, Gerday C.

Laboratory of Biochemistry, Institute of Chemistry B6, University of Liege, B-4000 Liege, Belgium.

Chloride is the allosteric effector of vertebrate pancreatic and salivary alpha-amylases and of the bacterial alpha-amylase from Alteromonas haloplanctis. Activation experiments of A. haloplanctis alpha-amylase by several monovalent anions show that a negative charge, not restricted to that of Cl-, is essential for the amylolytic reaction. Engineering of the chloride binding site reveals that a basic residue is an essential component of the site. The mutation K337R alters the Cl--binding properties, whereas the mutation K337Q produces an active, chloride-independent enzyme. Comparison of the Kd values for Cl- in three homologous alpha-amylases also indicates that the binding affinity is dependent on the chloride coordination mode by this basic residue. Analysis of substrate and chloride binding according to the allosteric kinetic model shows that the chloride effector is not involved in substrate binding. By contrast, the pH dependence of activity and experiments of chemical modifications and Ca2+ inhibition show that the chloride ion is responsible for the pKa shift of catalytic groups and interacts with active site carboxyl groups.

PMID: 8798613 [PubMed - indexed for MEDLINE]

2: Appl Environ Microbiol 1994 Sep;60(9):3096-104

Properties and active center of the thermostable branching enzyme from Bacillus stearothermophilus.

Takata H, Takaha T, Kuriki T, Okada S, Takagi M, Imanaka T.

Biochemical Research Laboratories, Ezaki Glico Co., Ltd., Osaka, Japan.

Although the branching enzyme (EC 2.4.1.18) is a member of the alpha-amylase family, the characteristics are not understood. The thermostable branching enzyme gene from Bacillus stearothermophilus TRBE14 was cloned and expressed in Escherichia coli. The branching enzyme was purified to homogeneity, and various enzymatic properties were analyzed by our improved assay method. About 80% of activity was retained when the enzyme was heated at 60 degrees C for 30 min, and the optimum temperature for activity was around 50 degrees C. The enzyme was stable in the range of pH 7.5 to 9.5, and the optimum pH was 7.5. The nucleotide sequence of the gene was determined, and the active center of the enzyme was analyzed by means of site-directed mutagenesis. The catalytic residues were tentatively identified as two Asp residues and a Glu residue by comparison of the amino acid sequences of various branching enzymes from different sources and enzymes of the alpha-amylase family. When the Asp residues and Glu were replaced by Asn and Gln, respectively, the branching enzyme activities disappeared. The results suggested that these three residues are the catalytic residues and that the catalytic mechanism of the branching enzyme is basically identical to that of alpha-amylase. On the basis of these results, four conserved regions including catalytic residues and most of the substrate-binding residues of various branching enzymes are proposed.

PMID: 7944355 [PubMed - indexed for MEDLINE]

3: Eur J Biochem 1994 Apr 15;221(2):649-54

Residues essential for catalytic activity of soybean beta-amylase.

Totsuka A, Nong VH, Kadokawa H, Kim CS, Itoh Y, Fukazawa C.

Genetic Engineering Laboratory, National Food Research Institute, Ibaraki, Japan.

To determine which amino acid residues are essential for the catalytic activity of soybean beta-amylase, deoxyoligonucleotide site-directed mutagenesis was employed against aspartyl, glutamyl, and cysteinyl residues located in highly conserved regions found in beta-amylase family to date. Both substitution of aspartic acid at position 101 and that of glutamic acid at position 186 of the enzyme by neutral and acidic amino acids, respectively, led to the complete elimination of activity, but did not induce any significant changes in circular dichroic spectra or the binding affinity for cyclomaltohexaose, a substrate analogue. Taking account of the results obtained here, the above two amino acid residues are involved in the catalytic site of soybean beta-amylase. The replacement of glutamic acid at position 345 decreased activity to below 6% of the non-mutant level, implying that this residue may also play a crucial role in beta-amylase activity, although it may not be involved at the catalytic site itself. In contrast, substitution of cysteinyl residue at position 95 by a serinyl residue led to a drastic reducing of the optimal temperature (from 50 degrees C to 30 degrees C), suggesting that this cysteinyl residue is responsible for the thermal stability of the enzyme.

PMID: 8174545 [PubMed - indexed for MEDLINE]

4: Eur J Biochem 1994 Apr 1;221(1):277-84

Domain B protruding at the third beta strand of the alpha/beta barrel in barley alpha-amylase confers distinct isozyme-specific properties.

Rodenburg KW, Juge N, Guo XJ, Sogaard M, Chaix JC, Svensson B.

Carlsberg Laboratory, Department of Chemistry, Copenhagen, Denmark.

alpha-Amylases belong to the alpha/beta-barrel protein family in which the active site is created by residues located at the C-terminus of the beta strands and in the helix-connecting loops extending from these ends. In the alpha-amylase family, a small separate domain B protrudes at the C-terminus of the third beta strand of the (beta/alpha)8-barrel framework. The 80% identical barley alpha-amylase isozymes 1 and 2 (AMY1 and AMY2, respectively) differ in substrate affinity and turnover rate, CaCl2 stimulation of activity, sensitivity to the endogenous 21-kDa alpha-amylase/subtilisin inhibitor, and stability at low pH. To identify regions that confer these isozyme-specific variations, AMY1-AMY2 hybrid cDNAs were generated by in vivo homologous recombination in yeast. The hybrids AMY1-(1-90)-AMY2-(90-403) and AMY1-(1-161)-AMY2-(161-403) characterized in this study contain the 90-residue and 161-residue N-terminal sequences, respectively, of AMY1 and complementary C-terminal regions of AMY2. AMY1-(1-90)-AMY2-(90-403) comprises the 60-amino-acid domain B of AMY2 and resembles this isozyme in sensitivity to alpha-amylase/subtilisin inhibitor and its low affinity for the substrates p-nitrophenyl alpha-D-maltoheptaoside, amylose and the inhibitor acarbose. Only AMY1-(1-161)-AMY2-(161-403) and AMY1, which both share domain B, are stable at low pH. However, AMY2 and both hybrid AMY species, but not AMY1, show maximum enzyme activity on insoluble blue starch at approximately 10 mM CaCl2. Domain B thus determines several functional and stability properties that distinguish the barley alpha-amylase isozymes.

PMID: 8168517 [PubMed - indexed for MEDLINE]

5: Biochim Biophys Acta 1993 Sep 3;1202(1):129-34

Change of substrate specificity by chemical modification of lysine residues of porcine pancreatic alpha-amylase.

Yamashita H, Nakatani H, Tonomura B.

Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Japan.

Lysine residues of porcine pancreatic alpha-amylase (PPA) were modified with trinitrobenzenesulfonate (TNBS). 6 out of 21 lysine residues were modified per PPA molecule. Amylase activity (hydrolysis of the alpha-1,4-D-glucoside bond) was decreased to about 50% of the native enzyme, as judged from the kcat value at pH 6.9 after the modification, whereas maltosidase activity (hydrolysis of p-nitrophenyl-alpha-D-maltoside producing p-nitrophenol and maltose) was increased to about 250%. The increase in maltosidase activity of the modified PPA was due to the increase in kcat, but not to the decrease in Km. Modification of PPA with five kinds of acid anhydrides also caused the same effect as TNBS, including the number of modified lysine residues. The degree of increase in maltosidase activity was fairly proportional to the volume of the incorporated modification reagent. A modification protection study in the presence of maltotriitol (G3OH), which protected two out of six modifiable lysine residues against modification, suggested that a lysine residue at the substrate-binding site contributes to the change of substrate specificity.

PMID: 8373816 [PubMed - indexed for MEDLINE]

6: Eur J Biochem 1993 Feb 1;211(3):899-902

Effect of mutation of an amino acid residue near the catalytic site on the activity of Bacillus stearothermophilus alpha-amylase.

Takase K.

Department of Molecular Biology, National Institute of Agrobiological Resources, Tsukuba, Japan.

Site-directed mutagenesis of a thermostable alpha-amylase from Bacillus stearothermophilus was performed to assess the role of amino acid residues near the catalytic site in catalysis. Asn329 is presumed to be adjacent to the proposed catalytic residue Asp331. Its mutation to Lys, which is found at the corresponding position in pullulanase, resulted in the loss of 99.7% of the activity, while the mutation to Asp or Val did not drastically reduce the activity. The mutation to Val altered the temperature/activity profile so that the activity was reduced to 25% of wild-type alpha-amylase at 60 degrees C but was over twofold greater at 5 degrees C. This effect could be ascribed to a decrease in the activation enthalpy by 32%. The mutation to Asp or Lys altered the pH/activity profile concomitant with possible changes in the ionization state of the groups introduced. These results show the feasibility of altering and possibly improving the enzyme activity by mutagenesis of residues near the catalytic groups.

PMID: 8436143 [PubMed - indexed for MEDLINE]

7: Biochem Biophys Res Commun 1992 Feb 28;183(1):286-91

Multi-functional roles of a histidine residue in human pancreatic alpha-amylase.

Ishikawa K, Matsui I, Honda K, Nakatani H.

National Chemical Laboratory for Industry, Ibaraki, Japan.

Functional roles of histidine residues at the active site in human pancreatic alpha-amylase were examined by protein engineering. Three histidine residues at 101, 201, and 299 were converted to asparagine residues, respectively. It was found that His201 played multi-functional roles concerning so many functions; substrate binding, control of optimum pH, change in substrate specificity,

activation by chloride ion, and inhibition by a proteinaceous inhibitor.

PMID: 1543499 [PubMed - indexed for MEDLINE]

8: J Bacteriol 1991 Oct;173(19):6147-52

Analysis of the active center of Bacillus stearothermophilus neopullulanase.

Kuriki T, Takata H, Okada S, Imanaka T.

Biochemical Research Laboratories, Ezaki Glico Co., Ltd., Osaka, Japan.

The active center of the neopullulanase from Bacillus stearothermophilus was analyzed by means of site-directed mutagenesis. The amino acid residues located in the active center of the neopullulanase were tentatively identified according to a molecular model of Taka-amylase A and homology analysis of the amino acid sequences of neopullulanse, Taka-amylase A, and other amylolytic enzymes. When amino acid residues Glu and Asp, corresponding to the putative catalytic sites, were replaced by the oppositely charged (His) or noncharged (Gln or Asn) amino acid residue, neopullulanase activities toward alpha-(1----4)- and alpha-(1----6)-glucosidic linkages disappeared. When the amino acids corresponding to the putative substrate-binding sites were replaced, the specificities of the mutated neopullulanases toward alpha-(1----4)- and alpha-(1----6)-glucosidic linkages were obviously different from that of the wild-type enzyme. This finding proves that one active center of neopullulanase participated in the dual activity toward alpha-(1----4)- and alpha-(1----6)-glucosidic linkages. Pullulan is a linear glucan of maltotriosyl units linked through alpha-(1----6)-glucosidic linkages. The production ratio of panose from pullulan was significantly increased by using the mutated neopullulanase which exhibited higher specificity toward the alpha-(1----4)-glucosidic linkage. In contrast, the production ratio of panose was obviously decreased by using the mutated neopullulanse which exhibited higher specificity toward the alpha-(1----6)-glucosidic linkage.

PMID: 1917847 [PubMed - indexed for MEDLINE]

9: Arch Biochem Biophys 1991 Aug 15;289(1):124-9

The pH dependence of the action pattern in porcine pancreatic alpha-amylase-catalyzed reaction for maltooligosaccharide substrates.

Ishikawa K, Matsui I, Honda K, Kobayashi S, Nakatani H.

National Chemical Laboratory for Industry, Ibaraki, Japan.

Porcine pancreatic alpha-amylase (EC 3.2.1.1; abbreviated PPA), which hydrolyzes alpha-D-(1,4) glucosidic bonds in starch and amylose, displays an optimum at pH 6.9 for the majority of substrates. The optimum pH, however, shifted to 5.2 for the hydrolysis of some low molecular substrates (Ishikawa, K., et al., 1990, Biochemistry 29, 7119-7123). Details of the substrate-dependent shift of the optimum pH in PPA were studied by use of a series of maltooligosaccharides with 14C-labeled reducing end glucose as substrates. The optimum pH for maltotriose was 5.2, whereas that for maltopentaose and maltohexaose was unchanged at pH 6.9. The pH profile for the intermediate size substrate maltotetraose showed abnormality; the apparent optimum pH was broadened between 5.5 and 6.5 and the bond cleavage pattern depended on pH, unlike that for the other substrates examined. These results were independent of either buffer systems or substrate concentration. Analyses of the hydrolysates of the maltooligosaccharides revealed that the shift of the optimum pH to the neutral region occurred only when the fifth subsite of PPA in the productive binding modes was occupied by a glucosyl residue of a substrate. The three-catalytic residue model of PPA deduced from the analysis of the hydrolysis of some modified maltooligosaccharides (p-nitrophenyl-alpha-D-maltoside, gamma-cyclodextrin,

maltopentaitol, and maltohexaitol) (Ishikawa, K., et al., 1990, Biochemistry 29, 7119-7123) was successfully adapted to the linear maltooligosaccharides used in this work. These results indicate that the different productive binding modes of the linear oligosaccharide substrates affect directly the catalytic power and the optimum pH of PPA.

PMID: 1898059 [PubMed - indexed for MEDLINE]

10: Biochemistry 1990 Jul 31;29(30):7119-23

Substrate-dependent shift of optimum pH in porcine pancreatic alpha-amylase-catalyzed reactions.

Ishikawa K, Matsui I, Honda K, Nakatani H.

National Chemical Laboratory for Industry, Ibaraki, Japan.

Porcine pancreatic alpha-amylase (EC 3.2.1.1, abbreviated as PPA) hydrolyzes alpha-D-(1,4) glucosidic bonds in starch and amylose at random, and the optimum pH for the substrates is 6.9. The optimum pH, however, shifted to 5.2 for the hydrolytic reaction of low molecular weight oligosaccharide substrates such as p-nitrophenyl alpha-D-maltoside, gamma-cyclodextrin, maltotetaitol, and maltopentaitol. The optimum pH for the oligosaccharides consisting of more than five glucose residues, such as maltopentaose and maltohexaitol, was 6.9. From the analysis of the hydrolysates, it was clear that the shift of the optimum pH occurred only when the fifth subsite of PPA in the productive binding modes was occupied by a glucosyl residue of the substrates. The value of Km was independent of pH between 4 and 10 but that of kcat was dependent on pH. The pH profiles of kcat for the above substrates did not fit a simple bell-shaped curve predicted by a two-catalytic-group mechanism. Instead, they were well analyzed theoretically by three pK values and two intrinsic kcat values. Enthalpy changes for the three pK's (4.90, 5.35, and 8.55 at 30 degrees C) were determined from the temperature dependence of pH profiles for maltopentaitol and maltohexaitol to be 0.0, 2.87, and 7.33 kcal/mol, respectively. These results indicate that productive binding modes of the substrates directly affect the catalytic function of the enzyme. From the present thermodynamic analysis and reported three dimensional structure at the active site of PPA [Buisson, G. (1987) EMBO J. 6, 3909-3916], one can assume that a histidyl residue (101, 201, or 299) acts as a proton donor and two carboxyl groups (Asp 197, Glu 233, or Asp 300) act as proton donors or acceptors, and the productive binding mode covering the fifth subsite changes configurations between the catalytic residues and the glucosidic bond hydrolyzed and modulates kinetic parameters depending on pH.

PMID: 2223766 [PubMed - indexed for MEDLINE]

11: J Biochem (Tokyo) 1986 Apr;99(4):1245-52

Inspection of active sites of human salivary alpha-amylase isozymes by means of non-reducing-end substituted maltooligosaccharides with 2-pyridylamino residue.

Omichi K, Ikenaka T.

The modes of action of four alpha-amylase isozymes, which were purified from human saliva, on p-nitrophenyl alpha-maltopentaoside (G5P), maltohexaitol (G6R), and their 2-pyridylamino derivatives, p-nitrophenyl O-6-deoxy-6-[(2-pyridyl)amino]-alpha-D-glucopyranosyl-(1----4)-O-alpha-D-glucopyranosyl-(1----4)-O-alpha-D-glucopyranosyl-(1----4)-O-alpha-D-glucopyranosyl-(1----4)-alpha-D-glucopyranoside (FG5P) and O-6-deoxy-6-[(2-pyridyl)amino]-alpha-D-glucopyranosyl-(1----4)-O-alpha-D-glucopyranosyl-(1----4)-O-alpha-D-glucopyranosyl-(1----4)-O-alpha-D-glucopyranosyl-(1----4)-O-alpha-D-glucopyranosyl-(1----4)-D- glucitol (FG6R) were examined at various pH values. No differences in their modes of action on the substrates was found. Irrespective of which enzyme was used, the

molar ratio of the hydrolysis products of G5P or G6R was almost constant at any pH examined. On the other hand, those of FG5P and FG6R varied with pH such that predominantly O-6-deoxy-6-[(2-pyridyl)amino]-alpha-D-glucopyranosyl-(1----4)-O-alpha-D-glucopyranosyl-(1----4)-D-glucose (FG3) was formed at high pH ranges, while the formation of O-6-deoxy-6-[(2-pyridyl)amino]-alpha-D-glucopyranosyl-(1----4)-O-alpha-D-glucopyranosyl-(1----4)-O-alpha-D-glucopyranosyl-(1----4)-D-gl ucose (FG4) increased at lower pH. The result indicates that the binding mode of FG5P or FG6R to the active sites of the enzymes changed with pH; namely, interactions between the 2-pyridylamino residue of the substrates and some amino acid residue(s) located in the active sites were influenced by pH.(ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 3486866 [PubMed - indexed for MEDLINE]

12: J Biochem (Tokyo) 1983 Apr;93(4):1195-201

Difference spectroscopic study of the interaction between soybean beta-amylase and substrate or substrate analogues.

Nitta Y, Kunikata T, Watanabe T.

1. In order to investigate the interactions between soybean beta-amylase [EC 3.2.1.2] and ligands (maltotriose as substrate, and maltose and alpha- and beta-cyclodextrins as inhibitors for the hydrolysis of maltoheptaose), the difference spectra were measured at 25 degrees C and pH 5.4, in 0.05 M acetate buffer. Each difference spectrum produced by these ligands showed a clear peak at 292-293 nm due to a tryptophan residue. In addition to this peak, the spectra of alpha- and beta-cyclodextrins showed a specific peak at 298-299 nm, and that of maltotriose showed a shoulder at 298 nm. 2. From the concentration dependency of the difference molar extinction delta epsilon, at 292-293 nm or at 298-299 nm, the dissociation constant of the enzyme-ligand complex, Kd, was evaluated for maltotriose, and alpha- and beta-cyclodextrins. For each ligand, the Kd values obtained at these two wavelengths were in good agreement with Michaelis constant, Km, or the inhibitor constant, Ki. The Kd value for maltose obtained from the titration of delta epsilon at 292 nm was also in good agreement with Ki. 3. Maltose produced a hydrophobic change in the environment of the tryptophan residue, while the interactions of maltotriose, and alpha- and beta-cyclodextrins with this enzyme caused an electrostatic change in the vicinity of the tryptophan residue in addition to the hydrophobic change. Since the signal at 298-299 nm was not found in the difference spectrum of maltose, this signal may be due to a tryptophan residue different from that which produces the signal at 292-293 nm. If both the signals are due to the same tryptophan residue, we must conclude that some conformational change is caused in the enzyme active site by the ligand binding.

PMID: 6190798 [PubMed - indexed for MEDLINE]

13: J Biochem (Tokyo) 1982 Sep;92(3):653-9

Kinetic study on chemical modification of taka-amylase A. I. Location and role of tryptophan residues.

Kita Y, Fukazawa M, Nitta Y, Watanabe T.

1. Five and four tryptophan residues in Taka-amylase A [EC 3.2.1.1] of A. oryzae (TAA) were modified with dimethyl(2-hydroxy-5-nitrobenzyl)-sulfonium bromide (K-IWS) in the absence and the presence of 15% maltose (substrate analog), respectively. Only one tryptophan residue was modified with dimethyl(2-methoxy-5-nitrobenzyl)-sulfonium bromide (K-IIWS) irrespective of the presence or absence of maltose. Kinetic parameters (molecular activity, k0, Michaelis constant, Km, and inhibitor constant, Ki) of the enzyme modified with K-IWS and K-IIWS were determined. The k0 value decreased with increase in the

number of modified residues, but Km and Ki values and the type of inhibition were not altered by the modification. 2. The fluorescence quenching reaction of TAA with N-bromosuccinimide (NBS) proceeded in three phases. The second-order rate constants of the three phases were determined to be (4.3 +/- 0.5) x 10(5) M-1 . s-1, (2.1 +/- 0.3) x 10(3) M-1 . s-1 and (1.7 +/- 0.2) x 10(2) M-1 . s-1, respectively. In the presence of maltose, the first phase was further separated into two phases with rate constants of (4.6 +/- 0.6) x 10(6) M-1 . s-1 and (6.9 +/- 1.1) x 10(4) M-1 . s-1, respectively. On the basis of the results, it is estimated that five out of nine tryptophan residues are accessible to the solvent and among them, two tryptophan residues are substantially exposed: one is located in the maltose binding site near the catalytic site (its modification affects the catalytic function), and the other exists on the enzyme surface far from the active site.

PMID: 6183254 [PubMed - indexed for MEDLINE]

14: Biochemistry 1976 May 4;15(9):1987-93

Identity and properties of the chloride effector binding site in hog pancreatic alpha-amylase.

Lifshitz R, Levitzki A.

The Cl- activated alpha-amylase from mammalian sources has been shown previously to possess one Cl- binding site per molecule (Levitzki, A., and Steer, M.L. (1974), Eur. J. Biochem. 41, 171). Upon binding of the Cl- effector the kcat of the amylolytic reaction is increased 30-fold whereas the affinity toward the substrate remains unchanged. In the study presented here we have identified the Cl- binding site as a single epsilon-amino group of lysine. The pK of the unique amino group was found to be 9.1, significantly lower than the pH of a free epsilon-amino group of lysine. This epsilon-NH2 group can be blocked by a 2, 4-dinitrophenyl group upon treating the enzyme with 2, 4-dinitrofluorobenzene at pH 7.9. The dinitrophenylamylase is devoid of Cl- binding capacity but retains its substrate binding capacity. The dinitrophenylamylase also possesses the basal amylolytic activity characteristic of the unmodified Cl- free enzyme, indicating that the catalytic machinery of the enzyme is not affected by dinitrophenylation. alpha-Limit dextrins and maltose which bind to the active site protect the enzyme against dinitrophenylation at lea-st as effectively as the Cl- effector. These observations indicate that the Cl- binding lysyl residue is close to the active site and, upon binding, the Cl- effector induces an enhancement in the catalytic efficiency.

PMID: 817737 [PubMed - indexed for MEDLINE]

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L1             1 9068-25-1/RN

=> d

L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2001 ACS
RN   9068-25-1  REGISTRY
CN   Mannosidase, exo-1,2-.alpha.- (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   .alpha.-1,2-Mannosidase
CN   .alpha.-Mannosidase I
CN   1,2-.alpha.-D-Mannosidase
CN   E.C. 3.2.1.113
CN   Exo-.alpha.-1,2-mannanase
CN   Exo-1,2-.alpha.-mannosidase
CN   Mannose-9 processing .alpha.-mannosidase
MF   Unspecified
CI   MAN
LC   STN Files:   AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, CA, CAPLUS, TOXLIT,
       USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
             156 REFERENCES IN FILE CA (1967 TO DATE)
               2 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
             157 REFERENCES IN FILE CAPLUS (1967 TO DATE)
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NiceZyme View of ENZYME: EC 3.2.1.113

Official Name	
Mannosyl-oligosaccharide 1,2-alpha-mannosidase.	
Alternative Name(s)	
Mannosidase 1A. Mannosidase 1B.	
Reaction catalysed	
Hydrolysis of the terminal 1,2-linked alpha-D-mannose residues in the oligo-mannose oligosaccharide Man(9)(GlcNAc)(2).	
Comments	
• Involved in the synthesis of glycoproteins.	
Cross-References	
Biochemical Pathways; map number(s)	S5
BRENDA	3.2.1.113
EMP/PUMA	3.2.1.113
WIT	3.2.1.113
KYOTO UNIVERSITY LIGAND CHEMICAL DATABASE	3.2.1.113
MEDLINE	Find literature relating to 3.2.1.113
SWISS-PROT	P53624, M121_DROME; P53625, M122_DROME; P33908, MA12_HUMAN; P45700, MA12_MOUSE; P31723, MA12_PENCI; P45701, MA12_RABIT; P32906, MNS1_YEAST;

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BRENDA:3.2.1.113

E.C. number	3.2.1.113 (BRENDA copyright notice)
Original Organism	**#1#** Rat (male <1, 2>; Sprague-Dawley <1>; Wistar <2>) <1, 2, 7, 16, 19, 21> **#2#** Rabbit <3, 15, 17> **#3#** Bovine (calf) <10> **#4#** Human <5> **#5#** Pig <14, 20> **#6#** Dog <21> **#7#** Mung bean <6, 9, 13> **#8#** Ricinus communis (castor bean) <18> **#9#** Plants <19, 21> **#10#** Spodoptera frugiperda (IPLB-SF-21AE, baculovirus-infected) <22> **#11#** Aspergillus oryzae <4, 8> **#12#** Aspergillus saitoi <11, 12> **#13#** Saccharomyces cerevisiae <24> **#14#** Bacillus sp. (M-90) <23>
Systematic name	1,2-alpha-Mannosyl-oligosaccharide alpha-D-
Recommended name	Mannosyl-oligosaccharide 1,2-alpha-mannosidase
Synonyms	Glycoprotein processing mannosidase I #1# <7> Mannose-9 processing alpha-mannosidase Exo-alpha-1,2-mannanase 1,2-alpha-Mannosidase alpha-1,2-Mannosidase Mannosidase, exo-1,2-alpha- Mannosidase 1B Mannosidase I #12# <12> More #7# (not identical with aryl-alpha-mannosidase) <6> (alpha1,2)-Mannosidase-I #10# <22> Man9-mannosidase #5# <14> Mannosidase 1A
CAS registration number	9068-25-1
Reaction	Hydrolysis of the terminal 1,2-linked alpha-D-mannose residues in the oligo-mannose oligosaccharide Man9(GlcNAc)2
Reaction type	Hydrolysis of O-glycosyl compounds
Substrates/products	S: More #1,3-5,7,8,11,12,14# (mannosidase IA and B are quite similar in substrate specificity, #1# <2>; pig liver enzyme substrate specificity resembles that of calf liver enzyme, #5# <14>; no hydrolysis of alpha1,6-linkages, #7,12# <6, 12> or alpha1,3-linkages, #12# <12>; no substrates are 3-O-alpha- and 6-O-alpha-D-mannobioses, #11# <4>; (Man)5 (GlcNAc)2-Asn (i.e. GPI, #4# <5>), #4,11# <4, 5>; GP III, #4# <5>; (Man)4-(GlcNAc)2,

#8# <18>; 4-methylumbelliferyl-alpha-mannoside, #5# <14, 20>; 1,2-alpha-D-mannobiitol, #14# <23>; little or no activity with 4-methylumbelliferyl-alpha-mannoside, aryl-alpha-D-mannosides, #3# <10>) <2, 4-6, 10, 12, 14, 18, 20, 23>

P: ?

- S: (Glc)1(Man)9(GlcNAc)2 + H2O #3# (removal of one alpha1,2-linked mannose) <10>

  P: ?

- S: alpha-D-Man-(1-2)-D-Man + H2O #11# <8>

  P: D-Mannose #11# <8>

- S: Mannobiose + H2O #12# <11>

  P: D-Mannose #12# <11>

- S: 2-O-alpha-D-Mannopyranosyl-D-mannose + H2O #11# <4>

  P: D-Mannose #11# <4>

- S: p-Nitrophenyl-alpha-D-mannoside + H2O #1# (mannosidase IB: poor substrate, mannosidase IA: no substrate, #1# <2>; not, #1-3,5,7,8,10-12,14# <4, 6, 10, 12, 14-16, 18, 20-23>) <2>

  P: ?

- S: Baker's yeast mannan + H2O #11,12,14# (releases 9.2% of total mannose, #11# <4>; alpha-1,2-linked side-chains, #14# <23>) <4, 8, 11, 23>

  P: ?

- S: (Glc)3(Man)9(GlcNAc)2 + H2O #3# (removal of one alpha1,2-linked mannose) <10>

  P: ?

- S: Ovalbumin glycopeptide + H2O #2,10# (glycopeptide IV, #2# <15>) <15, 22>

  P: ?

- S: High mannose chains of thyroglobulin and phytohemagglutinin-P #2# (thyroglobulin: 70% of alpha1,2-mannose residues accessible) <15>

  P: ?

- S: alpha-1,2-Linked mannotetraose + H2O #14# <23>

  P: ?

- S: GlcNAc-(Man)5GlcNAc + H2O #1# (poor substrate) <2>

  P: ?

- S: Manalpha(1-6)(Manalpha(1-3))Manalpha(1-6)(Manalpha(1-3))Manbeta(1-4)GlcNAc #1# (without alpha1,2-linked mannose residues: poor substrate, #1# <1, 2>) <1, 2>

  P: (Man)4GlcNAc + (Man)3GlcNAc + mannose #1# <2>

- S: Methyl-2-O-alpha-D-mannopyranosyl-alpha-D-mannopyranoside + H2O #2# <15, 17>

  P: ?

- S: Manalpha(1-2)Manalpha(1-6)(Manalpha(1-3))Manalpha(1-6)(Manalpha(1-2Manalpha (1-2))Manalpha(1-3))Manbeta(1-4)GlcNAcbeta(1-4)GlcNAc-peptide #1# <1>

  P: (Man)5(GlcNAc)-peptide + mannose #1# <1>

- S: Manalpha(1-2)Manalpha(1-3)Manbeta(1-4)GlcNAc + H2O #12# <12>

  P: ?

- S: (Manalpha1-2)nManalpha1-6(Manalpha1-3)Manalpha1-6(Manalpha1-3) Manbeta1-

4GlcNAcbeta1-4GlcNAc + H2O #12# <12>

P: Manalpha1-6(Manalpha1-3)Manalpha1-6(Manalpha1-3)Manbeta1-4GlcNAc beta1-4GlcNAc + mannose #12# <12>

- S: Manalpha(1-2)Manalpha(1-6)(Manalpha(1-2)Manalpha(1-3)Manalpha(1-6)(Manalpha(1-2)Manalpha(1-2)Manalpha(1-3)Manbeta(1-4)GlcNAcbeta(1-4)GlcNAc #1,3-5,11# (i.e. (Man)9(GlcNAc)2 #4# <5>; highly active on alpha(1-2) linked mannooligosaccharides, #5,11# <4, 14>; removes 3 of the 4 alpha1,2-linked mannosyl residues, #3,5# <10, 14, 20>; intact chitobiose core affects Man9-mannosidase specificity: reduction or removal of terminal N-acetylglucosamine residue increases hydrolytic susceptibility of the fourth alpha1,2-mannosyl-linkage, #5# <20>; enzyme from endoplasmic reticulum removes only 1 or 2 alpha1,2-linked mannose residues, #1# <21>) <4, 5, 10, 14, 20, 21>

  P: (Man)6(GlcNAc)2 + mannose #3,5# <10, 20>

- S: Pyridylamino derivative of (Man)9(GlcNAc)2 + H2O #8# <18>

  P: Pyridylamino derivative of (Man)5(GlcNAc)2 + mannose #8# (after 120 h incubation, via specific intermediates: overview) <18>

- S: Manalpha(1-2)Manalpha(1-6)(Manalpha(1-3))Manalpha(1-6)(Manalpha(1-2Manalpha(1-2))Manalpha(1-3))Manbeta(1-4)GlcNAc #1# <1, 16>

  P: ?

- S: Manalpha(1-2)Manalpha(1-6)(Manalpha(1-3))Manalpha(1-6)(Manalpha(1-2Manalpha(1-2))Manalpha(1-3))Manbeta(1-4)GlcNAcbeta(1-4)GlcNAc #3,5# <10, 14>

  P: ?

- S: Manalpha(1-2)Manalpha(1-6)(Manalpha(1-3))Manalpha(1-6)(Manalpha(1-2)Manalpha(1-3))Manbeta(1-4)GlcNA #1# (hydrolysis at 39% (mannosidase IB) or 67% (mannosidase IA) the rate of (Man)9GlcNAc hydrolysis, #1# <2>) <1, 2, 16>

  P: ?

- S: Manalpha(1-6)(Manalpha(1-3))Manalpha(1-6)(Manalpha(1-2)Manalpha(1-3))Manbeta(1-4)GlcNAcbeta(1-4)GlcNAc-Asn #1# (hydrolysis at 18-25% the rate of (Man)9GlcNAc hydrolysis, #1# <2>) <1, 2, 16>

  P: ?

- S: Manalpha(1-6)(Manalpha(1-3))Manalpha(1-6)(Manalpha(1-2)Manalpha(1-3))Manbeta(1-4)GlcNAcbeta(1-4)GlcNAc #4,11# (i.e. GPIV, #4,11# <4, 5>) <4, 5>

  P: (Man)5(GlcNAc)2-Asn + mannose #4,11# (i.e. GPI, #4# <5>; major product formed, 3.5 mol mannose/mol GP IV per h, #4# <5>) <4, 5>

- S: Manalpha(1-6)(Manalpha(1-3))Manalpha(1-6)(Manalpha(1-2)Manalpha(1-3))Manbeta(1-4)GlcNAc #5,8,10,12# (preferred substrate, #10# <22>; as in Taka-amylase A, #12# <11>; very poor substrate, #5# <14, 20>; pyridylamino derivative of Manalpha1-6(Manalpha1-3)Manalpha1-6(Manalpha1-2Man alpha1-3)ManbetaGlcNAc2, #8# <18>; not, #3# <10>) <11, 14, 18, 20, 22>

  P: (Man)5(GlcNAc)2 + mannose #8,10# (i.e. pyridylamino derivative of (Manalpha1-3)Manbeta1-4GlcNAcbeta1-4GlcNAc, #8# <18>) <18, 22>

- S: Manalpha(1-2)Manalpha(1-6)(Manalpha(1-2)Manalpha(1-3)Manalpha(1-6)(Manalpha(1-2)Manalpha(1-2)Manalpha(1-3)Manbeta(1-4)GlcNAc #1,5,7,13,14# (i.e. (Man)9GlcNAc, specific for alpha-1,2-linked mannose residues, #1,14# <1, 23>; best substrate, #1# <2>; removes all 4 alpha1,2-linked mannosyl residues, #1,7# <7, 13>; the terminal mannose on the middle antenna appears to be the most susceptible residue, #1# <16>) <1, 2, 7, 9, 13, 16, 20, 21, 23, 24>

  P: (Man)5GlcNAc + mannose #1,5,7,14# (and (Man)6GlcNAc, #1# <1>; after long-term

	incubation, #7# <9>; (Man)8GlcNAc, (Man)7GlcNAc and (Man)6GlcNAc after 4 h, (Man)8GlcNAc, (Man)7GlcNAc, (Man)6GlcNAc and (Man)5GlcNAc after 12 h incubation, #7# <9>) <1, 7, 9, 13, 20, 23> • S: Manalpha(1-6)(Manalpha(1-3))Manalpha(1-6)(Manalpha(1-3))Manbeta(1-4) GlcNAcbeta(1-4)GlcNAc #2,7# (i.e. Manalpha1-2Manalpha1-2-Manalpha1-3 (Manalpha1-6)ManbetaGlcNAc-betaGlcNAc, #2,7# <3, 6, 15>; or heptasaccharide I, #7# <6>; without alpha1,2-linked mannose residues: no substrate, #1# <16>) <3, 6, 15> P: (Man)4(GlcNAc)2 + (Man)3(GlcNAc)2 + mannose #7# <6> • S: Manalpha(1-2)Manalpha(1-6)(Manalpha(1-3))Manalpha(1-6)(Manalpha(1-2)Manalpha (1-3))Manbeta(1-4)GlcNAcbeta(1-4)GlcNAc #3,5# <10, 14> P: ? • S: Manalpha(1-2)Manalpha(1-6)(Manalpha(1-2)Manalpha(1-3))Manalpha(1-6)(Manalpha (1-2)Manalpha(1-2)Manalpha(1-3)Manbeta(1-4)GlcNAcbeta(1-4)GlcNAc-hexapeptide #5# (substrate specificity not influenced by peptide moiety, removes 3 mannose residues) <20> P: (Man)6(GlcNAc)2-hexapeptide + mannose #5# <20>
Natural substrates	More #1-4# (reaction during Asn-linked oligosaccharide biosynthesis, #1# <1>; involved in synthesis, #2# <3> and processing, #4# <5> of Asn-linked complex-type oligosaccharides of mammalian glycoproteins, #2,4# <3, 5>; involved in glycoprotein processing, #1,3,4# <5, 7, 10>) <1, 3, 5, 7, 10>
Specific activity (micromol/min/mg)	• 34600 #12# <12> • 12 #11# <8> • 0.0000036 #8# ((Man)6-GlcNAc as substrate) <18> • -999 #1-3,5,7# <2, 9, 10, 13-16>
Km-value (mM)	• 1.25 #12# {Manalpha1-2Manalpha1-3Manbeta1-4GlcNAc} <12> • 0.67 #12# {mannobiose} <11> • 0.6 #2# {methyl-mannopyranosyl-mannopyranoside} <17> • 0.57 #11# {2-O-alpha-D-mannopyranosyl-D-mannose} <4> • 0.55 #2# {GPIV from ovalbumin} <15> • 0.3 #13# {(Man)9GlcNAc} <24> • 0.26 #8# {(Man)6GlcNAc} <18> • 0.1 #1# {(Man)8GlcNAc} <1> • -999 #5# (kinetic constants of various substrate derivatives) <20>
pH-optimum	• 7 #14# <23> • 6.5 #2# (in the presence of soybean phosphatidylinositol) <3> • 6 #1,5,7,10# (mannosidase IA and B, #1# <2>) <2, 9, 14, 16, 22> • 6-6.2 #3# <10> • 6-6.5 #1# ((Man)8(GlcNAc)2-peptide as substrate) <1> • 5.5-6 #7,8# (Man6B as substrate, #8# <18>) <6, 13, 18> • 5.5-6.5 #2# (in the presence of egg yolk lyso-phosphatidylcholine) <3> • 5 #1,12# (Man(alpha1-2)Man as substrate, #1# <21>) <12, 21> • 5-6 #2# (in the presence of 10 mM CaCl2, #2# <15>) <15, 17>

	• 4.9-5.3 #11# (2-O-alpha-D-mannopyranosyl-D-mannose as substrate) • -999 #14# (pI: 3.6) <23>
pH-range	• 5.5-7 #1,3# (about half-maximal activity at pH 5.5 and 7, #1,3# <1, 10>; (Man)8(GlcNAc) 2-peptide as substrate, #1# <1>) <1, 10> • 5.5-7.5 #2# (about half-maximal activity at pH 5.5 and 7.5, in the presence of soybean phosphatidylinositol) <3> • 5.3-6.6 #10# (about half-maximal activity at pH 5.3 and 6.6) <22> • 5.2-6.7 #5# (about half-maximal activity at pH 5.2 and 6.7) <14> • 5.2-7.3 #2# (about half-maximal activity at pH 5.2 and 7.3, in the presence of egg yolk lyso-phosphatidylcholine) <3> • 4.8-6.8 #7# (about half-maximal activity at pH 4.8 and 6.8) <9> • 4-5.8 #11# (about half-maximal activity at pH 4 and 5.8, 0.1 M acetate buffer) <4>
Temperature-optimum (deg.C)	• 40 #8# (assay at) <18> • 37 #1-5,7,10-12# (assay at) <1-6, 10, 12-17, 22> • 30 #12# (assay at) <11>
Cofactors/prosthetic groups	• More #2# (no activation by negatively charged phospholipids, dibutyryl-phosphocholine) <3> • Phosphatidylcholines #2# (activation, with acyl-chains of different lengths, above C-4) <3> • Phosphatidylethanolamine #2# (activation, in the presence of 3.5 mM Triton X-100) <3> • Sphingomyelin #2# (activation, in the presence of 3.5 mM Triton X-100) <3> • Triton X-100 #1,2,7# (requirement, #1# <16>; activation (together with zwitterionic phospholipids, #2# <3>), #2,7# <3, 9, 13>; 0.1%, #7# <9, 13>; 0.12%, no activation at 0.07% and below, #1# <16>) <3, 9, 13, 16> • Zwitterionic phospholipids #2# (activation, required for solubilized and purified enzyme, independent of acyl chain length or degree of saturation, an ordered lipid structure of either micelles or bilayers, mixed micelles together with Triton X-100) <3>
Metal ions/salts	• Ca2+ #2,5,7,8,10# (requirement, #2# <17>; activation, #2,5,7,8,10# <6, 9, 13-15, 18, 22>; most effective cation, #5# <14>; slight, #8# <18>; 5 mM, #7# <13>; microsomal enzyme, #7# <6>; mechanism, #2# <17>; not, #14# <23>) <6, 9, 13-15, 17, 18, 22> • Cd2+ #11# (activation, 1 mM, inhibits at 10 mM) <4> • Co2+ #7# (slight activation) <9> • Cu2+ #7# (slight activation) <9> • Divalent cations #3# (requirement, not, #12# <12>) <10> • Fe2+ #7# (slight activation) <9> • Mg2+ #5,7# (activation, #5,7# <13, 14>; less effective than Ca2+, #5# <14>; slight, #7# <13>; not, #11# <4>) <13, 14> • Mn2+ #7# (slight activation) <9, 13> • Zn2+ #5# (activation, less effective than Ca2+) <14>
Inhibitors	• 1,4-Dideoxy-1,4-imino-D-mannitol #1# (in vitro and in vivo, #1# <7>; not, #1,7,9# <9, 13, 19>) <7> • 1-Cyclohexyl-3-(2-morpholinyl-4-ethyl)carbodiimide/glycine methylester #12# <11>

- 1-Deoxymannojirimycin #1-3,5,7,8,10# (mannose analog, in vivo and in vitro, #1,2# <17, 19>; reversible (not by Ca2+, #2# <17>), #1# <16>; rat liver enzyme, #1# <19>; strong, #5# <20>; not (ER-enzyme, #1# <21>), #1,14# <21, 23>) <9, 10, 13-22>
- Ag2+ #11# (not, #12# <11>) <4>
- alpha1,2-Linked oligosaccharides #7# <9, 13>
- alpha1,3-Linked oligosaccharides #7# <9, 13>
- Ba2+ #11# (10 mM) <4>
- BAPTA #2# (i.e. 1,2-bis(2-aminophenoxy) ethane N,N,N',N'-tetraacetic acid) <15>
- Basic sugar analogs of mannose #5# (strong) <14>
- Cd2+ #8,11# (strong, #8# <18>; 10 mM, activation at 1 mM, #11# <4>) <4, 18>
- Co2+ #1,2,11,12# (weak, #1# <1>) <1, 4, 11, 15-17>
- Cu2+ #1,3,5,7,8,11,12# (strong, #7,8# <6, 18>; 10 mM, #11# <4>) <1, 4, 6, 10, 11, 14, 16, 18>
- D-Mannono-gamma-lactone #11# <4>
- D-Mannonolactam amidrazone #1# (mannosidase inhibitor, MDCK-cells: in vivo and in vitro) <21>
- EDTA #1-3,7,8,11-14# (weak, #1,12# <1, 11>; divalent cations, #13# <24>; Ca2+, #1,2,7# <6, 9, 13, 15, 21>; Mg2+ (slightly, #1# <21>; not, #2,7# <9, 15>), #3# <10>; Sr2+ (slightly), #1# <21>; Zn2+ (not, #1,2# <15, 21>), #3# <10> or Mn2+ (less efficiently, #3# <10>; not, #1,2# <15, 21>) restores, #1-3,7,13# <6, 9, 10, 13, 15, 21, 24>; not Cu2+, #1# <21>; Co2+, #1,2# <15, 21>; Ni2+ or Ba2+, #2# <15>) <1, 4, 6, 9-11, 15, 16, 18, 21, 23, 24>
- EGTA #2# (i.e. ethylene glycol-bis[beta-aminoethylether]-N,N,N',N'-tetraacetic acid) <15>
- Fe2+ #11,12# <4, 11>
- Fe3+ #8,11# (strong, #8# <18>) <4, 18>
- Hg2+ #8,11# (strong) <4, 18>
- Iodoacetic acid #11,12# (monoiodoacetic acid, 1 mM, weak, #12# <11>) <4, 11>
- Kifunensine #1,9# (plant and animal Golgi mannosidase I, #1,9# <19>; mannosidase I, not ER-enzyme from rat liver, #1# <21>) <19, 21>
- Mannosamine #7# (not, #12# <11>) <9, 13>
- Mannosyl-mannose disaccharide #7# (in decreasing order of efficiency: alpha1,2, alpha 1,3 and alpha1,6-mannosylmannose) <6>
- Methyl-alpha-mannoside #12# (not, #11# <4>) <11>
- Mn2+ #2,12# <11, 15, 17>
- More #1,2,5-12# (mannosidase IA and B are quite similar in response to inhibitors, #1# <2>; no inhibition by L-cysteine, #11# <4>; mannose, alpha1,6-linked mannose oligosaccharide, castanospermine, deoxynojirimycin, 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine, #7# <9, 13>; 4-nitrophenyl-alpha-mannoside, mannitol, L-mannono-1,4-lactone, PMSF, #12# <11>; Ca2+, #1# <16>; Mg2+, #1,2# <15-17>; swainsonine (little or not, #5# <14>), #1,2,5,7-10,14# <9, 13-15, 18-20, 22, 23>; mannostatin A, #1,6# <19, 21> or N,N-dimethyl-1-deoxymannojirimycin (little or not, #5# <14>), #5# <14, 20>) <2, 4, 9, 11, 13-22>
- N-5-Carboxypentyldeoxymannojirimycin #3,5# (strong, #5# <20>) <10, 20>
- N-Methyl-deoxymannojirimycin #1,3,5# <10, 14, 19>
- Na+ #11# (1 mM, weak, not at 10 mM) <4>
- Ni2+ #3# <10>

	• p-Chloromercuriphenylsulfonic acid #1# <16> • PCMB #2# (Ca2+ protects, #2# <15>; not, #12# <11>) <15> • Phosphatidylinositol #2# (CaCl2 or NaCl restores) <15> • Sn2+ #8# (strong) <18> • Ti4+ #8# (strong) <18> • Tris #1,2,8# (in vivo and in vitro, #2# <17>) <16-18> • Tris/maleate #1# <1> • Zn2+ #2,7,8,11# (strong, #7# <6>; reversible by Ca2+, #2# <17>) <4, 6, 15, 17, 18>
Source tissue	• Cell #13# <24> • Cell suspension culture #6,10# (MDCK-cells, #6# <21>) <21, 22> • Cotyledons #8# <18> • Culture filtrate #12# <11> • Culture fluid and mycelium #12# (commercially available under the name of Morushin) <12> • Culture supernatant #14# <23> • Hypocotyls #7# <6> • Liver #1-3,5,6# <1-3, 7, 10, 14-17, 19-21> • Liver metastases of colon adenocarcinoma #4# <5> • Madin-Darby canine kidney cells #1# (influenza virus-infected) <21> • Mycelium #12# <12> • Seedlings #7,8# <6, 9, 18> • Taka-diastase #11# (enzyme-product from Sankyo Co., Ltd.) <4, 8>
Localisation	• Cytosol #1,7# (2 enzymes: a membrane-bound microsomal and a soluble cytoplasmic one, #7# <6>; rat liver, #1# <21>) <6, 21> • Endoplasmic reticulum #1,3,5# (rat liver, #1# <21>) <10, 20, 21> • Golgi membranes #1,9,10# (cis-Golgi, #1,9# <19>) <1, 2, 16, 19, 22> • Membrane-bound #1-7,9# (2 enzymes: a membrane-bound microsomal and a soluble cytoplasmic one, #7# <6>) <1-3, 5, 6, 9, 10, 13-17, 21> • Microsomes #1-7,9# (2 enzymes: a membrane-bound microsomal and a soluble cytoplasmic one, #7# <6>; trans-membrane protein with cytosolic domain, #5# <14>) <3, 5, 6, 9, 10, 13-15, 17, 21> • Soluble #1,7# (2 enzymes: a membrane-bound microsomal and a soluble cytoplasmic one, #7# <6>; rat liver, #1# <21>) <6, 21>
Purification	• #1# (partial <1, 2, 7>; mannosidase IA <16>; enzyme from endoplasmic reticulum <21>) <1, 2, 7, 16, 21> • #10# <22> • #11# (affinity chromatography with baker's yeast mannan gel <8>) <4, 8> • #12# <12> • #13# (recombinant enzyme) <24> • #2# (partial, solubilization requires non-ionic detergents) <3> •

	#3# <10> #4# (partial) <5> #5# (affinity chromatography on immobilized N-5-carboxypentyl-1-deoxymannojirimycin) <14> #6# (partial) <21> #7# (partial <6>; affinity chromatography on mannan and mannosamine-Sepharose <9>) <6, 9, 13> #8# (partial) <18>
Molecular Weight	460000 #7# (mung bean, microsomal enzyme, gel filtration, presumably in a micelle or aggregate containing detergent, phospholipid or other protein) <6> 380000 #14# (Bacillus sp.) <23> 230000 #1# (rat, PAGE) <16> 65000 #5# (pig, detected in freshly prepared crude microsomal extracts using polyclonal antibodies, during purification the native enzyme, MW 65000, loses a membrane-spanning domain without losing its catalytic activity) <14> 49200 #2# (rabbit, sedimentation analysis) <15> 49000 #11# (Aspergillus oryzae, gel filtration) <4> 40000 #2# (rabbit, gel filtration) <15> 39000 #7# (mung bean, cytosolic enzyme, gel filtration) <6> -999 #2,12# (amino acid composition) <11, 15>
Subunits	? #3,10,12# (x * 56000, bovine, SDS-PAGE <10>; x * 63000, Spodoptera frugiperda, SDS-PAGE <22>; x * 64000, Aspergillus saitoi, SDS-PAGE <11>) <10, 11, 22> Dimer #14# (2 * 190000, Bacillus sp.) <23> Monomer #2,5# (1 * 49000, pig, SDS-PAGE <14, 20>; 1 * 52000, rabbit, SDS-PAGE, under reducing and non-reducing conditions <15>) <14, 15, 20> Tetramer #1# (4 * 57000-58000, rat, SDS-PAGE) <16>
Posttranslational modification	Glycoprotein #1,2,12# (19.4% neutral and 3.9% amino sugars, #12# <11>; 0.88% hexoses, #1# <16>) <11, 15, 16>
pH-stability	5 #2# (above, stable) <15>
Temperature stability (deg.C)	50 #1,11# (90 min, about 60-70% loss of activity, #1# <1>; 1 h, in 0.1 M acetate buffer, pH 5, 10% loss of activity, #11# <4>; mannosidase IB less stable than IA, #1# <2>) <1, 2, 4> 37 #1# (24 h, in 0.1 M sodium acetate buffer, pH 5.8, 30% loss of activity) <2> -999 #1# (mannosidase IA and B differ in thermostability) <2>
General stability	#5# Enzyme loses a membrane-spanning domain during purification which does not alter its catalytic properties <20> #1# Mannosidase IA and B differ in sensitivity to storage in liver cytoplasmic extracts, no interconversion between mannosidase IA and IB during cellulose phosphate chromatography <2> #5# Native enzyme protein is highly susceptible to proteolytic cleavage, not by glucopeptidase F <14>
Storage stability	#2# -20°C, in 10 mM HEPES/NaOH, pH 7.5, 2 mM MgCl2, 0.1% Nonidet-P40, 0.2 M NaCl, less than 10% loss of activity within 3 months <15>

- #12# -20°C, in 10 mM sodium acetate buffer, pH 5, with or without 1 mM CaCl2, at least 4 months <12>
- #1# 0-4°C, in 10 mM potassium phosphate buffer, pH 7.2, 0.5% Triton X-100, 4-6 months <16>
- #7# 0-4°C, in buffer with 10% glycerol, purified enzyme, a few days, partially purified enzyme, at least 2 weeks <9>
- #1# 2-4°C, in 10 mM potassium phosphate buffer, pH 7.2, 1% Triton X-100, 20% loss within 4 weeks <2>
- #1# 4°C, 1 mg protein/ml, in 50 mM phosphate buffer, pH 6.5, 5 mM MgCl2, 0.1% Triton X-100, 30 days <1>
- #12# 4°C, in 10 mM sodium acetate buffer, pH 5, with or without 1 mM CaCl2, at least 4 months <12>
- #7# 4°C, in buffer with 10% glycerol, a few days, at early stage of purification at least 2 weeks <13>
- #11# 4°C, pH 4.5-6, at least 6 months <4>

References

<1> Tabas, I., Kornfeld, S.: Purification and characterization of a rat liver Golgi alpha-mannosidase capable of processing asparagine-linked oligosaccharides:: J. Biol. Chem., 254; 11655-11663 (1979)

<2> Tulsiani, D.R.P., Hubbard, S.C., Robbins, P.W., Touster, O.: :: J. Biol. Chem., 253; 3660-3668 (1982)

<3> Forsee, W.T., Springfield, J.D., Schutzbach, J.S.: Effect of phospholipids on alpha-1,2-mannosidase activity:: J. Biol. Chem., 257; 9963-9967 (1982)

<4> Yamamoto, K., Hitomi, J., Kobatake, K., Yamaguchi, H.: Purification and characterization of 1,2-alpha-mannosidase of Aspergillus oryzae:: J. Biochem., 91; 1971-1979 (1982)

<5> Chandrasekaran, E.V., Savila, M., Nixon, D., Mendicino, J.: Purification and properties of alpha-D-mannose:beta-1,2-N-acetylglucosaminyl-transferases and alpha-D-mannosidases from human adenocarcinoma:: Cancer Res., 44; 4059-4068 (1984)

<6> Forsee, W.T.: Characterization of microsomal and cytosolic alpha-1,2-mannosidases from mung bean hypocotyls:: Arch. Biochem. Biophys., 242; 48-57 (1985)

<7> Palamarczyk, G., Mitchell, M., Smith, P.W., Fleet, G.W., Elbein, A.D.: 1,4-Dideoxy-1,4-imino-D-mannitol inhibits glycoprotein processing and mannosidase:: Arch. Biochem. Biophys., 243; 35-45 (1985)

<8> Tanimoto, K., Nishimoto, T., Saitoh, F., Yamaguchi, H.: Improved purification of Aspergillus oryzae 1,2-alpha-mannosidase by using mannan gel affinity chromatography:: J. Biochem., 99; 601-604 (1986)

<9> Szumilo, T., Kaushal, G.P., Hori, H., Elbein, A.D.: :: Plant Physiol., 81; 383-389 (1986)

<10> Schweden, J., Legler, G., Bause, E.: Purification and characterization of a neutral processing mannosidase from calf liver acting on (Man)9(GlcNAc)2 oligosaccharides:: Eur. J. Biochem., 157; 563-570 (1986)

<11> Shigematsu, Y., Tsukahara, K., Tanaka, T., Takeuchi, M., Ichishima, E.: :: Curr. Microbiol., 13; 43-46 (1986)

<12> Kobata, A., Amano, J.: Alpha-mannosidases I and II from Aspergillus saitoi:: Methods Enzymol., 138; 779-785 (1987) (review)

<13> Kaushal, G.P., Elbein, A.D.: Glycoprotein processing enzymes of plants:: Methods Enzymol., 179; 452-475 (1989) (review)

<14> Schweden, J., Bause, E.: Characterization of trimming Man9-mannosidase from pig liver. Purification of a catalytically active fragment and evidence for the transmembrane nature of the intact 65 kDa enzyme:: Biochem. J., 264; 347-355 (1989)

<15> Forsee, W.T., Palmer, C.F., Schutzbach, J.S.: Purification and characterization of an alpha-1,2-mannosidase involved in processing asparagine-linked oligosaccharides:: J. Biol. Chem., 264; 3869-3876 (1989)

<16> Tulsiani, D.R.P., Touster, O.: Mannosidase IA from rat liver Golgi membranes:: Methods Enzymol., 179; 446-451 (1989)

<17> Schutzbach, J.S., Forsee, W.T.: Calcium ion activation of rabbit liver alpha 1,2-mannosidase:: J. Biol. Chem., 265; 2546-2549 (1990)

<18> Kimura, Y., Yamaguchi, O., Suehisa, H., Tagaki, S.: In vitro hydrolysis of oligomannose-type sugar chains by an alpha-1,2-mannosidase from microsomes of developing castor bean cotyledons:: Biochim. Biophys. Acta, 1075; 6-11 (1991)

<19> Elbein, A.D.: Glycosidase inhibitors: inhibitors of N-linked oligosaccharide processing:: FASEB J., 5; 3055-3063 (1991) (review)

<20> Bause, E., Breuer, W., Schweden, J., Roeser, R., Geyer, R.: Effect of substrate structure on the activity of Man9-mannosidase from pig liver involved in N-linked oligosaccharide processing:: Eur. J. Biochem., 208; 451-457 (1992)

<21> Pan, Y.-T., Kaushal, G.P., Papandreou, G., Ganem, B., Elbein, A.D.: D-mannonolactam amidrazone. A new mannosidase inhibitor that also inhibits the endoplasmic reticulum or cytoplasmic alpha-mannosidase:: J. Biol. Chem., 267; 8313-8318 (1992)

<22> Ren, J., Bretthauer, R.K., Castellino, F.J.: Purification and properties of a Golgi-derived (alpha 1,2)-mannosidase-I from baculovirus-infected lepidopteran insect cells (IPLB-SF21AE) with preferential activity toward mannose6-N-acetylglucosamine2:: Biochemistry, 34; 2489-2495 (1995)

<23> Maruyama, Y., Nakajima, T., Ichishima, E.: A 1,2-alpha-D-mannosidase from a Bacillus sp.: purification, characterization, and mode of action:: Carbohydr. Res., 251; 89-98 (1994)

<24> Lipari, F., Herscovics, A.: Production, purification and characterization of recombinant yeast processing alpha 1,2-mannosidase:: Glycobiology, 4; 697-702 (1994)